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A Laser-Guided Spinal Cord Displacement Injury in Adult Mice

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Abstract

Mouse models are unique for studying molecular mechanisms of neurotrauma because of the availability of various genetic modified mouse lines. For spinal cord injury (SCI) research, producing an accurate injury is essential, but it is challenging because of the small size of the mouse cord and the inconsistency of injury production. The Louisville Injury System Apparatus (LISA) impactor has been shown to produce precise contusive SCI in adult rats. Here, we examined whether the LISA impactor could be used to create accurate and graded contusive SCIs in mice. Adult C57BL/6 mice received a T10 laminectomy followed by 0.2, 0.5, and 0.8 mm displacement injuries, guided by a laser, from the dorsal surface of the spinal cord using the LISA impactor. Basso Mouse Scale (BMS), grid-walking, TreadScan, and Hargreaves analyses were performed for up to 6 weeks post-injury. All mice were euthanized at the 7th week, and the spinal cords were collected for histological analysis. Our results showed that the LISA impactor produced accurate and consistent contusive SCIs corresponding to mild, moderate, and severe injuries to the cord. The degree of injury severities could be readily determined by the BMS locomotor, grid-walking, and TreadScan gait assessments. The cutaneous hyperalgesia threshold was also significantly increased as the injury severity increased. The terminal lesion area and the spared white matter of the injury epicenter were strongly correlated with the injury severities. We conclude that the LISA device, guided by a laser, can produce reliable graded contusive SCIs in mice, resulting in severity-dependent behavioral and histopathological deficits.

Keywords: behavioral test; contusion; mouse; SCI; tissue displacement

Introduction

Spinal cord injury (SCI) is a devastating incident with permanent paralysis and disability outcomes. According to the National Spinal Cord Injury Statistical Center in 2018, a total of 17,700 new SCI cases are estimated to occur each year in the United States.[1] There have been no effective interventions available for treating SCI. SCI can be classified into several types including contusion, compression, laceration, sudden acceleration-deceleration, and complete transection.[2] The contusion injury is the most common type of SCI seen clinically.[3] Developing accurate, reproducible, and clinically relevant SCI models is crucial to unraveling the pathophysiology of SCI and developing specific treatments.

Since the first SCI device invented by Allen and coworkers in 1911,[4] several contusion SCI models have been introduced with their inherent strengths and weaknesses.[5–12] We previously reported the development of the Louisville Injury System Apparatus (LISA) impactor (Louisville, KY), which produced reliable and reproducible SCIs in adult rats.[13] The LISA impactor has several unique features.[13] First, LISA detects the ''0'' point of the spinal dorsal surface by a laser sensor that allows a precise determination of the displacement distance of the injury. Second, LISA applies a novel stabilizer to fixate the spinal column at the level of injury, which minimizes movement of the spinal cord at the time of impact.[13] Because transgenic and knockout mice are more available than rats, reliable mouse SCI models have become ideal tools to study cellular and molecular mechanisms of SCI as well as to develop new treatments for it.

To produce a consistent SCI, the laminectomy must be at the same level, and the impactor should always hit the midline if a bilateral injury is desired. Additionally, reliable parameters of an impact device are required to produce precise, graded contusive SCI. An injury device depends on several parameters, including force, weight, height, and displacement. In the present study, we report the use of the LISA impactor to determine whether graded SCIs could be produced and whether such graded SCIs resulted in

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severity-dependent behavior and histological outcome changes along with injury-associated spontaneous recoveries.

Methods

Animals

Adult female C57BL/6 mice ($n=36$, 10 weeks old, 18–22 g) were purchased from Charles River Inc. (Wilmington, MA). Mice were randomly divided into four groups: sham ($n=6$) and injury displacements of 0.2 mm ($n=10$), 0.5 mm ($n=10$), and 0.8 mm ($n=10$). Sham-operated mice received the same surgical procedures, including laminectomy, as the SCI groups although they did not sustain an SCI. All animal care, handling, and surgery were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council) and the Guidelines of the Institutional Animal Care and Use Committee of Indiana University School of Medicine.

Surgery

A contusive SCI on mice was produced at the 10th thoracic vertebral level (T11/12 spinal cord segments) using the LISA impactor as described.[14] Prior to surgery, the mouse was anesthetized with intraperitoneal injection of ketamine (87.7 mg/kg)/xylazine (12.3 mg/kg). A midline skin incision was made over the lower thoracic spine. The muscles were separated from the T9-11 spinous processes and laminae, and the mouse was placed into the U-shaped customized vertebral stabilizer. The T10 facets were fixed bilaterally with the stabilizer, and the T10 laminectomy was performed to expose the dura over the spinal cord. The pressure of the nitrogen tank that controls the impact tip was adjusted to 18 psi or 124 kPa. The U-shaped stabilizer with the mouse was loaded onto the stage of the LISA and the height of the dura/spinal cord was adjusted directly under the impactor that was monitored by the laser beam. We reported a detailed video demonstration of the step-by-step procedures for producing a contusive SCI in mice using LISA.[14] After injury, the U-shaped stabilizer was detached from the stage, and the mouse was removed from the stabilizer. The injury area was checked and, if present, bleeding was stopped. The muscles and skin were sutured in layers with 3-0 silk surgical suture (Henry Schein, Melville, NY). The injured mouse received subcutaneous (SC) injection of buprenorphine (0.01–0.05 mg/kg every 8–12 h for 48 h) and 1 mL saline for hydration. The mouse was placed in a temperature- and humidity-controlled cage until it recovered from anesthesia. Soft food and water were provided during recovery. Manual bladder expression was performed twice daily until reflex bladder emptying was established.

Behavioral assessments

Four behavior assessments including Basso Mouse Scale (BMS), grid-walking, TreadScan, and Hargreaves tests were employed. Prior to surgery, each mouse was trained in the open field 4 min for BMS and on the grid 3 min for grid-walking per day for up to 3 days. Baseline scores of the four assessments were recorded. Methods for individual behavior assessments are described in the next sections.

BMS locomotor test. The BMS locomotor test was performed weekly up to 5 weeks post-SCI according to a method published previously.[15] Each mouse was placed in an open field (diameter 42 inch) and observed for 4 min by two observers who were blinded to the experimental groups. The movements were scored based on the joint movement, weight support, plantar stepping, paw position, coordination, and trunk and tail control following the standard guide.[15] The scores ranged from 9 to 0 and represent normal movement to complete paralysis, respectively.[15] BMS was recorded before injury and at 1, 7, 14, 21, 28, 35, and 42 days post-injury.

Grid-walking. The grid-walking test assesses the ability of a mouse to place its paws on the rungs of a grid accurately during spontaneous exploration. An elevated metal rectangle grid (12×36 inch^2 with each grid cell 0.5×0.5 inch^2) and 16 inches above a table top was used for the hindlimb drop error test. Each mouse was placed in the center of the grid for 3 min. A one-foot drop error was counted when the hindlimb paw was entirely dropping through the grid with all toes and heel below the grid surface. The total number of foot drop errors and total number of steps were recorded by video. Two observers who were blinded to the experiment analyzed the video and counted the steps.[16–18] The grid-walking test was performed on the day before surgery and on days 21 and 35 post-SCI. Only those mice with consistent plantar stepping (BMS ≥5) were tested on the grid.

TreadScan. Gait analysis using TreadScan (CleverSys Inc, Reston, VA) allows highly sensitive, noninvasive evaluation of extremities through a forced locomotion (treadmill) to indicate pathophysiological conditions occurring following SCI.[19] TreadScan recording and analysis followed a reported description.[19] Briefly, each mouse walked on the transparent treadmill for 20 sec with a speed of 7 cm/sec before injury and at 6 weeks post-injury. A high speed digital video camera recorded all the movements. The TreadScan software identified each individual paw profile, such as initial foot contact, stance time, print length, track width, and toe spread. For each 20 sec session, four to six consecutive step cycles of consistent walking underwent video analysis. Only the mice with consistent plantar stepping (BMS ≥5) were tested on the treadmill.

Hargreaves test. The noxious thermal stimulus test was used with the Hargreaves apparatus which was set at 30% intensity 20 sec cutoff time to avoid paw skin damage according to our previous protocol.[20] Briefly, each mouse was placed on the prewarmed (34°C) glass surface (IITC Life Science, Woodland Hills, CA), and a heat source stimulated the hindlimb plantar paw under the glass surface until the paw withdrew from the noxious thermal stimulus. The latency time (sec) of paw withdraw was recorded. Each paw was tested three times.[20, 21] The Hargreaves test was performed before injury and on day 21 post-injury. Only those mice with consistent plantar placing of the paw (BMS ≥3) were tested.

Histological assessments

A 3 cm segment of the spinal cord containing the injury epicenter was dissected out and removed after perfusion with 0.1 M phosphate-buffered saline (PBS) (30 mL) and 4% paraformaldehyde (PFA) (30 mL) on day 45 post-injury. The spinal cord segments were transferred to 4% PFA overnight and then transferred to 30% sucrose in 0.01 M PBS (pH 7.4) for 1 week.[22] One centimeter of the cord segment at the injury epicenter was subsequently embedded with OCT embedding solution (Fisher HealthCare, Waltham, MA) and cut with a cryostat into 25-μM-thick cross-sections and mounted on frosted slides in five identical sets, eight slides per set, and eight sections per slide. One set of the sections/slides was stained with cresyl violet eosin for lesion area measurement, and another set of sections was used for Luxol Fast Blue staining for myelin quantification. Areas of lesion and spared white matter (WM) were measured using Olympus BX60 microscope (Olympus, Tokyo, Japan) equipped with a Neurolucida System (MicroBrightField, Colchester, VT).[23] Briefly, at the epicenter, the area of the lesion, spared WM, and the total cord were outlined and measured. The lesion area (%) was calculated as lesion area divided by the cord area; the spared WM (%) was calculated as spared WM area divided by the cord area. All values were reported as mean ± standard error of the mean (SEM) (sham $n=6$, 0.2 mm $n=10$, 0.5 mm $n=8$, 0.8 mm $n=4$).

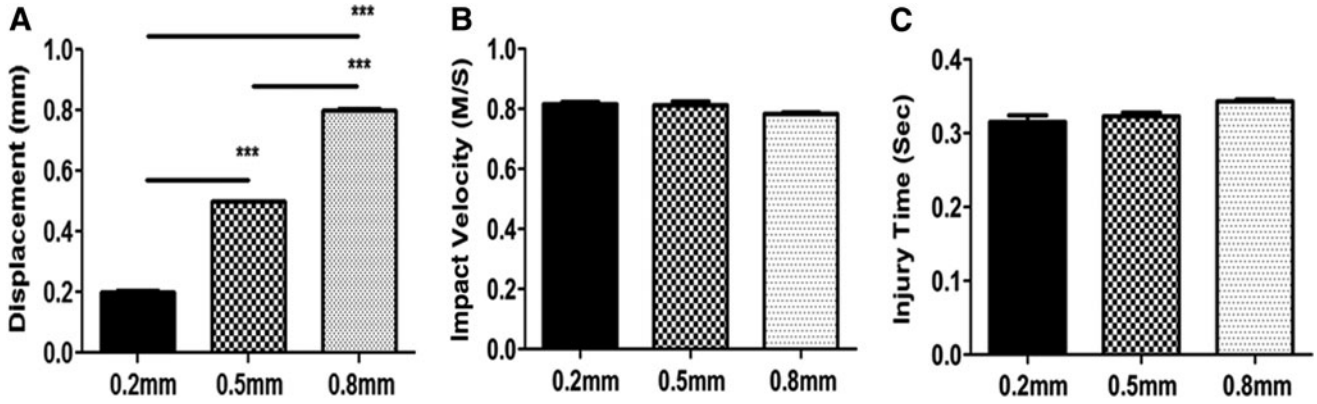

FIG. 1. The parameters of injury produced by the Louisville Injury System Apparatus (LISA) impactor. **(A)** Tissue displacement. **(B)** Impact velocity. **(C)** Injury duration time. All values are mean ± SEM (0.2 mm, $n = 10$; 0.5 mm, $n = 10$; 0.8 mm, $n = 10$. ***$p < 0.0001$.)

Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0 software (GraphPad, La Jolla, CA). Data were presented as mean ± SEM values. One way or two way ANOVA or repeated measures as appropriate followed by Tukey multiple comparison test were used to determine statistical significance among multiple groups with one factor, or multiple groups with two factors. XY analyses were obtained by correlation and linear regression. The difference was considered significant when $p < 0.05$.

Results

Injury parameters

LISA produced accurate and consistent cord displacements in three injury groups: 0.201 ± 0.0026 mm (mean ± SEM), 0.500 ± 0.0014 mm, and 0.801 ± 0.0024 mm, representing mild, moderate, and severe injuries to the cord ($p < 0.0001$, Fig. 1A). The velocity and duration times of the three groups were consistent (Fig. 1B, C). These parameters indicated that the graded contusive injuries produced by the LISA impactor in mice were reliable and reproducible.

Behavior assessments

Within the 6 week period when behavioral assessments were performed, 6 out of the 10 mice impacted at 0.8 mm died, 3 mice died within 1 day, and 3 died within 2 days. Two of the 10 mice impacted at 0.5 mm died within 2 days. None of the mice died in the 0.2 mm injury group or the sham group.

Open field locomotion. The hindlimb locomotor function of mice was tested with BMS up to 6 weeks post-injury (Fig. 2). Mice

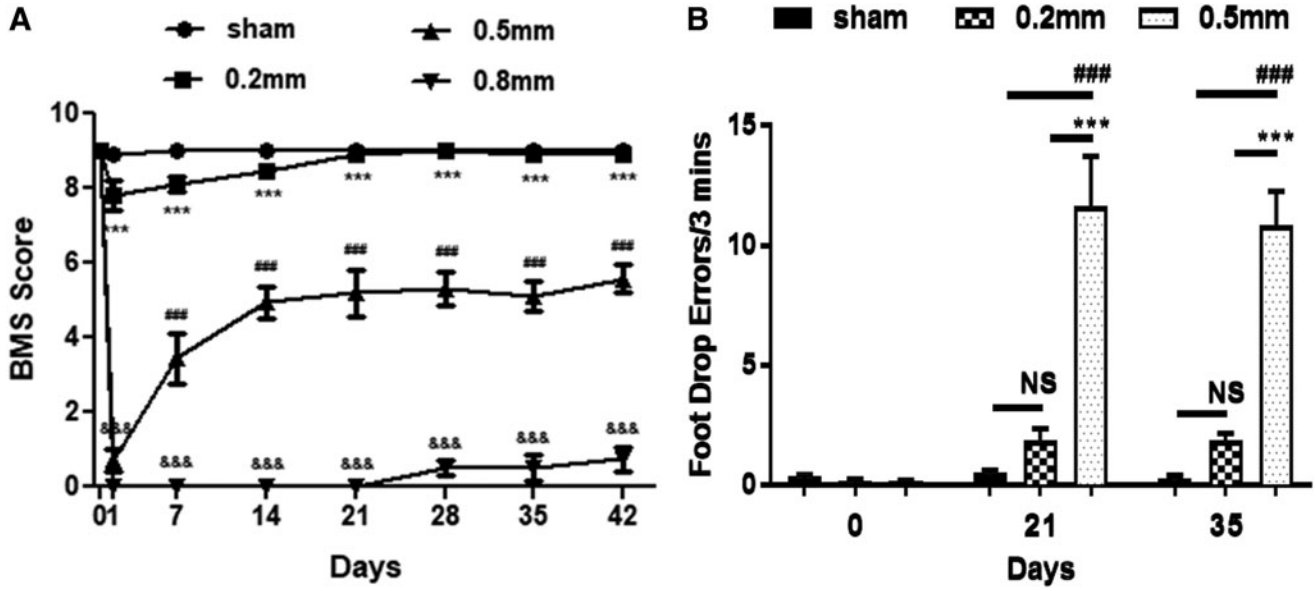

FIG. 2. Spontaneous locomotor recovery up to 6 weeks post-injury. **(A)** Basso Mouse Scale (BMS) locomotor scores showed a significant difference between the 0.2 mm and 0.5 mm (***$p < 0.001$), 0.2 mm and 0.8 mm (&&&$p < 0.001$), and 0.5 mm and 0.8 mm (###$p < 0.001$) injury groups. **(B)** The grid-walking test showed hindlimb foot drop errors among different groups. The foot drop errors were slightly increased in the 0.2 mm group and significantly increased in the 0.5 mm group compared with sham (###$p < 0.001$). There was a significant difference between the 0.5 mm and the 0.2 mm injury groups (***$p < 0.001$). Sham, $n = 6$; 0.2 mm, $n = 10$; 0.5 mm, $n = 8$; 0.8 mm, $n = 4$. All values are mean ± SEM.

in the 0.2 mm injury group showed slight deficits of trunk instability on days 1, 7, and 14 after SCI, and then recovered to the baseline level between 3 and 6 weeks. There was no difference between the sham and the 0.2 mm injury group 3 weeks post-injury. The mice in the 0.5 mm injury group demonstrated an intermediate degree of functional loss and spontaneous recovery. On the 1st day post-SCI in the 0.5 mm injury group, the hindlimbs of these mice were completely paralyzed and then gradually recovered over the 6 week survival period. The final mean BMS score of the 0.5 mm injury group at the 6th week was 5.56 ± 1.0 (mean ± SEM). In the 0.8 mm injury group, mice sustained a severe SCI, and only 4 of 10 mice survived. The hindlimbs remained paralyzed up to 21 days in the mice that survived an SCI at 0.8 mm. After day 28, slight ankle movements were observed. The final BMS score of the 0.8 mm injury group was 0.75 ± 0.55 (mean ± SEM). A two way ANOVA analysis revealed that there were statistically significant differences between the 0.2 and 0.5 mm injury groups (***$p < 0.001$), between the 0.2 and 0.8 mm injury groups (&&&$p < 0.001$), and between the 0.5 and 0.8 mm injury groups (###$p < 0.001$) throughout the testing period (Fig. 2A). BMS scores were significantly decreased as the tissue displacement increased from 0.2 to 0.8 mm (Fig. 2A).

Grid-walking. The foot drop errors were counted on both hindlimbs over a wire mesh (0.5×0.5 inch^2 grid spaces, 12×36 inch^2 total areas). Mice that had demonstrated frequent or consistent plantar stepping in the open field (BMS scores ≥ 5) were tested on the wire mesh. In the 0.8 mm injury group, mice only showed ankle movements and the BMS scores were <5. Therefore, they were excluded from the test. Mice in the 0.2 and 0.5 mm groups were tested at 21 and 35 days. For the 0.2 mm injury group, foot drop errors were higher than those of the sham group at 21 and 35 days. However, there were no statistically significant differences between the two groups. For the 0.5 mm injury group at days 21 and 35 post-injury, foot drop errors were significantly higher than those of the sham (###$p < 0.001$) and 0.2 mm groups (***$p < 0.001$, Fig. 2B).

TreadScan. At 6 weeks post-injury, mice demonstrated frequent or consistent plantar stepping in the open field (BMS scores ≥ 5) using the TreadScan.[19] Mice that sustained severe SCI in the 0.8 mm injury group with BMS scores ≤5 were excluded from the test. The gait characteristics were analyzed by parameters of stance time, rear-track width, hindlimb print length, and toe spread (Fig. 3A). The average stance time gradually decreased by 20% ($p < 0.05$) and 48% ($p < 0.001$) in the 0.2 mm and 0.5 mm groups compared with sham (Fig. 3B). In the 0.5 mm group, the average stance time significantly decreased by 35% compared with the 0.2 mm group ($p < 0.01$, Fig. 3B). The average rear-track width and print length were decreased in the 0.2 mm group, but there was no statistical difference compared with sham. However, they significantly decreased by 47% and 46% in the 0.5 mm group compared with sham ($p < 0.001$, Fig. 3C, D). Compared with the 0.2 mm group, the average rear-track width and print length were significantly reduced by 43% ($p < 0.001$) and 35% ($p < 0.01$), respectively, in the 0.5 mm group (Fig. 3C, D). The average toe spread was significantly decreased in the 0.2 mm group by 27% ($p < 0.05$) and in the 0.5 mm group by 59% ($p < 0.001$) compared with sham (Fig. 3E). In the 0.5 mm group, the average toe spread significantly decreased by 44% compared with the 0.2 mm group ($p < 0.01$, Fig. 3E). These results indicated that the gait loss was dependent on the increase of tissue displacement.

In order to evaluate the hindlimb coordination, we analyzed the parameters of trunk and hindlimb coordination (Fig. 4A). The maximum lateral deviation (LatD max, the farthest the foot deviated from the body axis) showed a significant decrease in the 0.5 mm group compared with the 0.2 mm (25% decrease) and sham (29% decrease) groups ($p < 0.001$, Fig. 4B). There was no statistically significant difference between the 0.2 mm and sham groups. However, there was a significant decrease in the minimum lateral deviation (LatD min, the closest the foot approached the body axis) in the 0.2 mm (25% decrease) and 0.5 mm (63% decrease) groups compared with sham as the injury severity increased ($p < 0.001$, Fig. 4C). Compared with the 0.2 mm group, the LatD min significantly decreased by 51% in the 0.5 mm group ($p < 0.001$, Fig. 4C). The minimum longitudinal deviation (LongD min, closest distance during the stance that the foot attained relative to the short body or waist axis) significantly increased in the 0.5 mm group by 76%

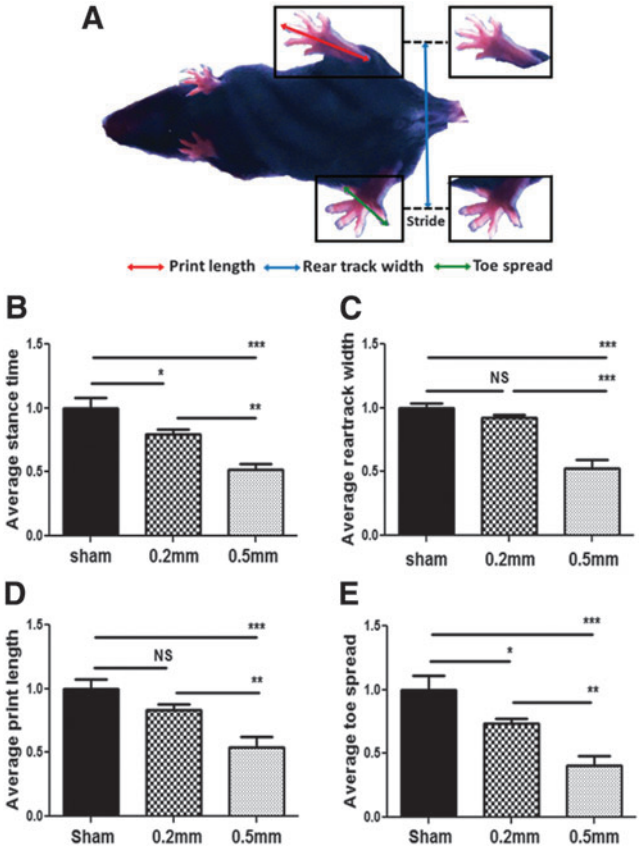

FIG. 3. Gait analysis using TreadScan. **(A)** Representative mouse image illustrating the selected gait parameters. **(B)** The average stance time decreased by 20% ($p < 0.05$) in the 0.2 mm and 48% ($p < 0.001$) in the 0.5 mm injury groups compared with sham. In the 0.5 mm group, the average stance time significantly decreased by 35% compared with the 0.2 mm group ($p < 0.01$). **(C, D)** The average rear-track width and average print length showed no difference in the 0.2 mm group, but significantly decreased by 47% and 46% in the 0.5 mm group as compared with sham group, respectively ($p < 0.001$). Compared with the 0.2 mm group, the average rear-track width and print length were significantly reduced by 43% ($p < 0.001$) and 35% ($p < 0.01$), respectively, in the 0.5 mm group. **(E)** The average toe spread was significantly decreased in the 0.2 mm group by 27% ($p < 0.05$) and in the 0.5 mm group by 59% ($p < 0.001$) compared with sham. In the 0.5 mm group, the average toe spread significantly decreased by 44% compared with the 0.2 mm group ($p < 0.01$). Sham, $n = 6$; 0.2 mm, $n = 10$; 0.5 mm, $n = 8$. Values are mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Color image is available online at www.liebertpub.com/neu

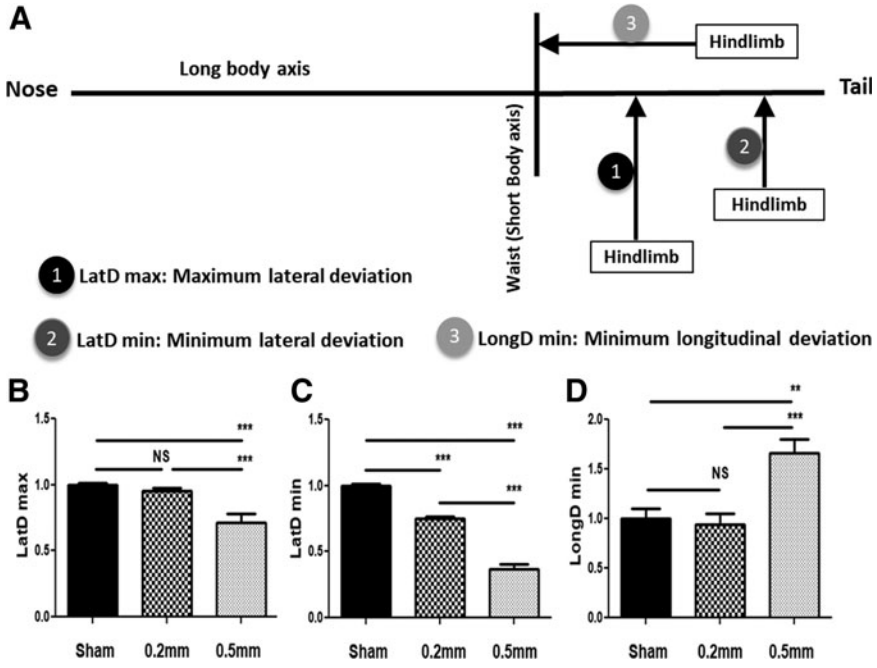

FIG. 4. Interlimb coordination using TreadScan. **(A)** Schematic representation of mouse interlimb coordination. **(B)** Average maximum lateral deviation (LatD max) showed no difference in the 0.2 mm group and significantly decreased by 29% ($p < 0.001$) in the 0.5 mm injury group compared with sham. The LatD max showed a significant decrease in the 0.5 mm group compared with the 0.2 mm group (25% decrease, $p < 0.001$). **(C)** The average minimum lateral deviation (LatD min) was significantly decreased in the 0.2 mm (25%) and 0.5 mm groups (63%) as compared with sham ($p < 0.001$). Compared with the 0.2 mm group, the LatD min significantly decreased by 51% in the 0.5 mm group ($p < 0.001$). **(D)** The minimum longitudinal deviation (LongD min) significantly increased in the 0.5 mm group by 76% ($p < 0.001$) and 66% ($p < 0.01$) compared with the 0.2 mm and sham groups, respectively. There was no statistically significant difference between the 0.2 mm and sham groups. Sham, $n = 6$; 0.2 mm, $n = 10$; 0.5 mm, $n = 8$. Values are mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$.

($p < 0.001$) and 66% ($p < 0.01$) compared with the 0.2 mm and sham groups, respectively (Fig. 4D). There was no statistically significant difference between the 0.2 mm and sham groups.

Hargreaves test. Mice in the 0.8 mm group were excluded because of their inability to place the plantar paw on the surface. At 3 weeks post-injury, the withdrawal latencies of the hindpaws were significantly increased in both the 0.2 and 0.5 mm injury groups compared with sham ($p < 0.05$, $p < 0.01$, respectively) (Fig. 5). However, there was no statistically significant difference between the 0.2 and 0.5 mm injury groups (Fig. 5).

Histopathological assessments

We assessed the lesion area and spared white matter at 45 days post-SCI. No detectable injury or myelin staining changes were identified in the 0.2 mm injury group. In the 0.5 and 0.8 mm injury groups, lesion areas were clearly seen (Fig. 6A, upper row) and the difference in lesion areas between the two groups was statistically significant ($p < 0.001$, Fig. 6B). Likewise, myelin loss was clearly seen in the 0.5 and 0.8 mm injury groups and was injury-severity dependent. The percentage of spared white matter was progressively decreased at the lesion epicenter as the injury severity increased (Fig. 6A, bottom row), and the differences among different groups were statistically significant ($p < 0.001$, Fig. 6C).

Correlations

To evaluate how injury severities affect the parameters of behavior, lesion area, and spared white matter, the Pearson *R*s of correlation and linear regression were run with GraphPad Prism 7.0. The data showed that the terminal BMS score ($R^2 = 0.99$, $p < 0.0001$, Fig. 7A), minimum lateral deviation (LatD min) ($R^2 = 0.94$, $p < 0.0001$, Fig. 7D), lesion area ($R^2 = 0.87$, $p < 0.0001$, Fig. 7E), and spared white matter ($R^2 = 0.83$, $p < 0.0001$, Fig. 7F)

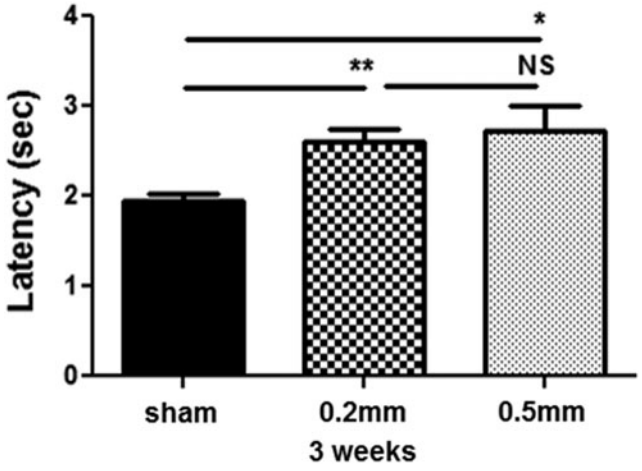

FIG. 5. Hargreaves test on the 3rd week post-SCI. The withdrawal latencies of the hindpaws were significantly increased in both the 0.2 and 0.5 mm injury groups compared with sham ($p < 0.05$, $p < 0.01$, respectively). However, there was no significant difference between the 0.2 mm and 0.5 mm injury groups. Sham, $n = 6$; 0.2 mm, $n = 10$; 0.5 mm, $n = 8$. Values are mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

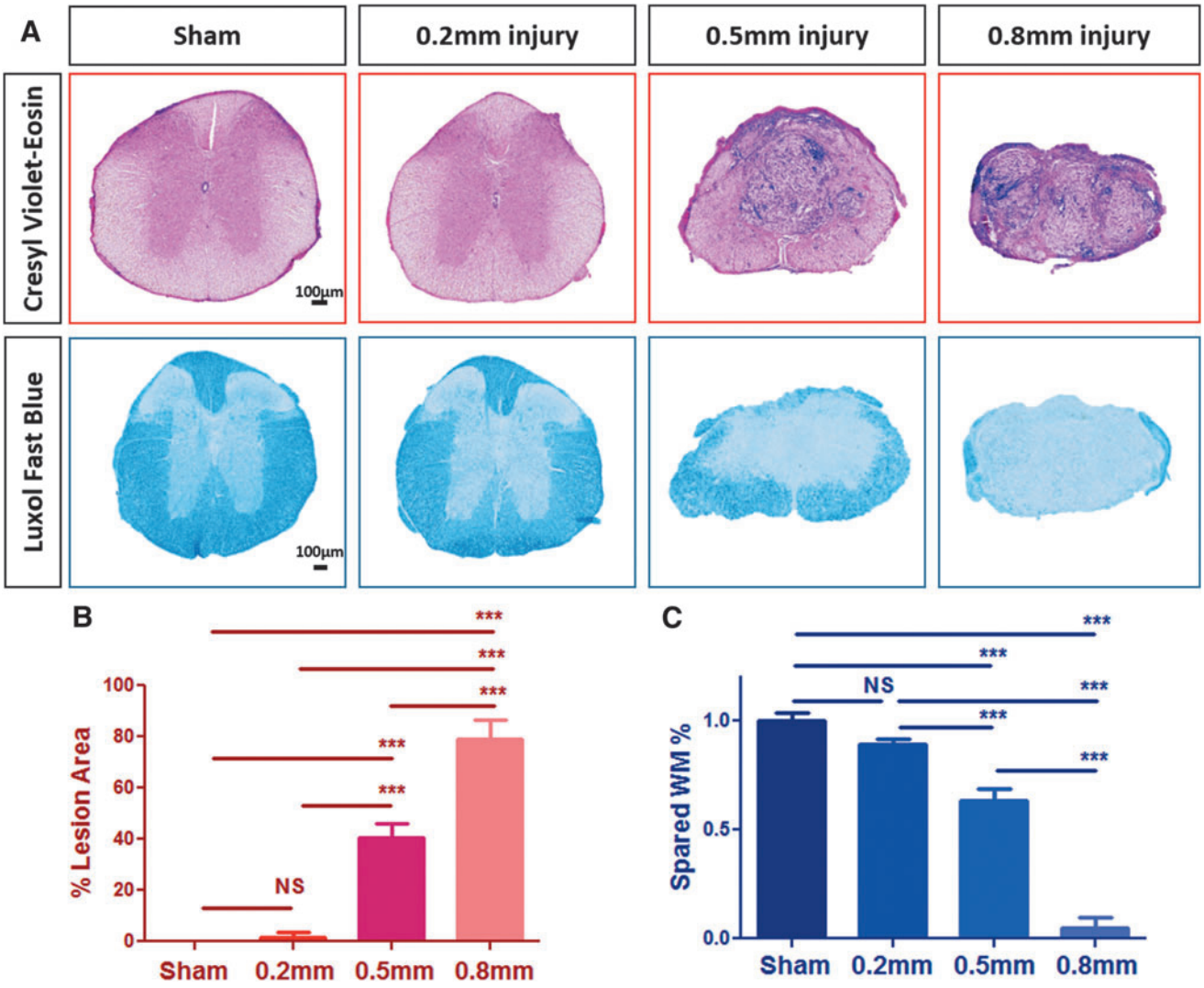

FIG. 6. Lesion area and spared white matter measured at the injury epicenter. **(A)** Cresyl violet eosin staining showed increased lesion areas as the injury severity increased (upper row). Luxol Fast Blue staining demonstrated decreased spared myelin areas as the injury severity increased (lower row). **(B)** Comparison of percent lesion area among different groups. **(C)** Comparison of percent spared white matter (WM) area among different groups. Sham, $n=6$; 0.2 mm, $n=10$; 0.5 mm, $n=8$; 0.8 mm, $n=4$. All values are mean ± SEM. ***$p<0.001$.

were strongly correlated with tissue displacement. The foot drop error ($R^2=0.73$, $p<0.0001$, Fig. 7B) and stance time ($R^2=0.63$, $p<0.0001$, Fig. 7C) also correlated significantly with tissue displacement.

Discussion

The contusion spinal cord injury is the most common type of SCI seen clinically, and the velocity of the impact is usually >1.0 m/sec.[3,11] In order to study the mechanism of pathophysiology and therapeutic inventions, developing reliable contusion animal models is necessary.[2] Many SCI contusion impactors have been developed. To date, the most widely used devices are New York University (NYU)/Multicenter Animal Spinal Cord Injury Studies (MASCIS),[5,6] the Ohio State University (OSU) Electromagnetic SCI Device (ESCID), and the Infinite Horizon (IH) impactor.[8,10] The NYU/MASCIS impactor is a weight drop model in which the amount of weight and height can be adjusted to produce mild, moderate, and severe SCIs on rats.[24–26] The different velocities (0.33–0.9 m/sec) and heights of weight drops determine the severity of injury.[5,27,28] The ESCID impactor is a tissue displacement device that produces a contusion severity based on the depth of injury. The maximum velocity of impact of the ESCID is 0.148 m/sec.[29] The IH impactor uses impact force to generate a gradable SCI with a maximum velocity of 0.13 m/sec.[7] Our model, the LISA impactor, is a relatively new model producing a contusive SCI based on tissue displacement.[13] While employing a similar tissue displacement mechanism as used by the ESCID, our model offers the following unique features. First, we use a mouse spine stabilizer to clamp vertebral facets at the level of injury, avoiding all movement artifacts that interfere with the accuracy of injury. Second, the noncontact guiding technique using a laser beam allows us to precisely measure the surface or zero point on the targeted spinal cord. This method creates a highly accurate displacement injury model. Third, the laser beam determines the midpoint of the injury, avoiding the lateralization of the injury. Finally, the impact velocity

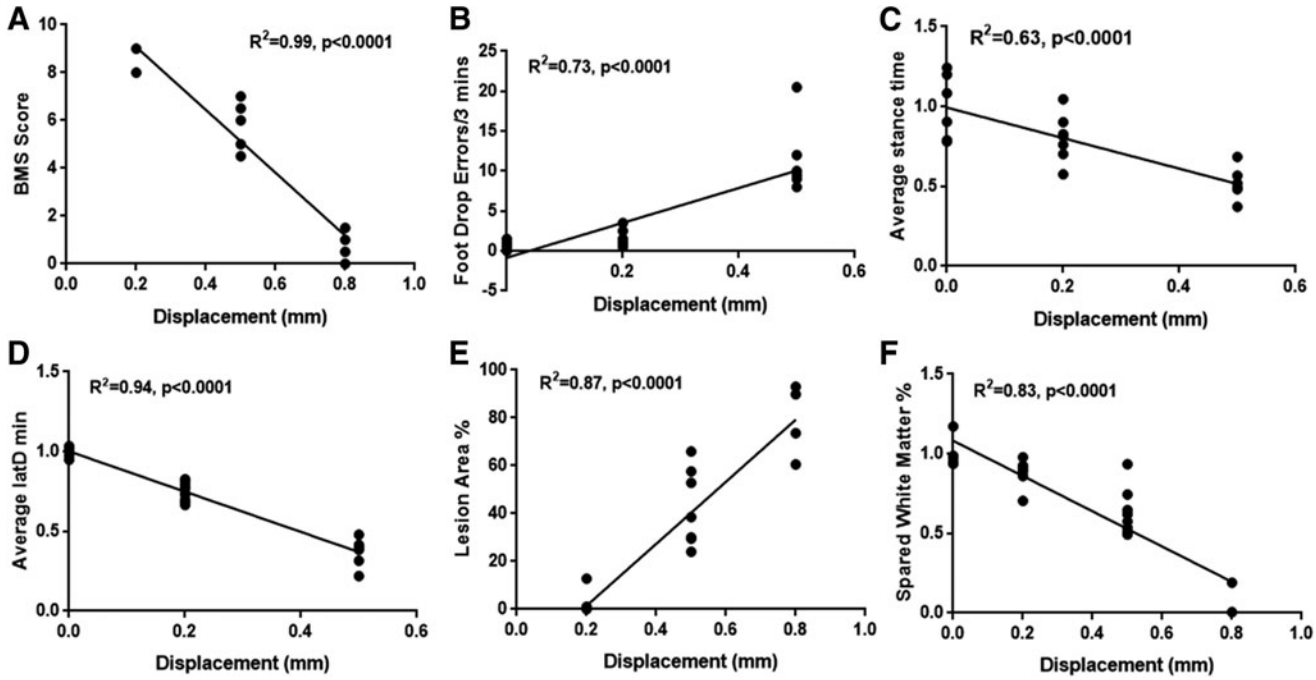

FIG. 7. Correlations of displacement with behavioral and histological parameters at 6 weeks post-spinal cord injury (SCI). Correlation between displacement and **(A)** Basso Mouse Scale (BMS) score, **(B)** foot drop errors, **(C)** average stance time, **(D)** average minimum lateral deviation (latD min), **(E)** percentage of lesion area, and **(F)** spared white matter area. 0.2 mm, $n=10$; 0.5 mm, $n=8$; 0.8 mm, $n=4$.

of our device is adjustable between 0.5 and 2 m/sec, which is the fastest among available injury devices and most clinically relevant.[13] These factors allow the LISA device to make precise, reproducible, and graded SCIs at a high-impact velocity. Because of the availability of more transgenic or gene-deficient species, the mouse LISA model could be a valuable tool to study cellular and molecular mechanisms and therapeutic inventions for SCI.[30–33]

In this study, the severities of SCI were determined by tissue displacements using a laser sensor. Among different injury modalities, the distance of displacement was the most stable parameter to be controlled and measured in producing an SCI. In our model, the variabilities of injury displacement were ±0.0025 mm in the 0.2 mm group (10 mice), ±0.0014 mm in the 0.5 mm group (8 mice), and ±0.0024 mm in the 0.8 mm groups (4 mice). These parameters of tissue displacement, produced by the LISA impactor, were reliable and highly correlated with behavioral and histological results.

In 2006, Basso and coworkers developed a valid locomotor rating scale, the BMS, for mice after SCI.[15] Using this scale, we demonstrated that the three displacement groups showed significant differences. At 6 weeks post-SCI, the BMS scores were 8.9 ± 0.32, 5.6 ± 1.05, and 0.75 ± 0.65 for 0.2 mm, 0.5 mm, and 0.8 mm displacement injuries, respectively, representing mild, moderate, and severe injuries in mice. There is a strong correlation between BMS scores and injury displacements.

In this study, we used the TreadScan gait analysis system to compare the normal and contusion-injured mice, as was reported by Beare and coworkers.[19] We demonstrated significant differences among groups in several hindlimb parameters after graded displacement injuries produced by the LISA. These included gait (average stance time, print length, rear-track width, and toe spread) and coordination (average LatD Max, LatD min, and LongD min). Among them, the average stance time, toe spread, and LatD min were highly correlated with the injury severities. We also found significant differences in average stance time, toe spread, and LatD min between the sham and 0.2 mm displacement groups with the TreadScan. Interestingly, these differences were not observed using the BMS, indicating that the TreadScan system is more sensitive than the BMS in measuring gait and coordinated functional differences between normal animals and those that had sustained a mild SCI.

The grid-walking (foot drop error) is a sensitive test for evaluating the sensorimotor coordination of the four limbs and has been used in many neurological diseases models, such as ischemic stroke,[34] somatosensory cortex lesion,[35] pyramidotomy,[36] and SCI.[16,17] Our data showed that foot drop errors of the hindlimbs were significantly different between the 0.2 mm and 0.5 mm injury groups. However, there was no statistically significant difference between the sham and 0.2 mm injury groups. These data indicated that mild and moderate injuries could be distinguished by the grid-walking test. Similarly, there was a significant correlation between foot drop errors and the magnitude of injury.

In 1988, Hargreaves and coworkers introduced a new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia.[21] They injected carrageenan in the plantar surface of one paw to produce inflammatory pain, and used the Hargreaves method to successfully detect the paw withdrawal latency changes corresponding to a decreased thermal nociceptive threshold.[21] Some studies showed that after SCI, the thermal nociception threshold (latency) was decreased, producing hyperalgesia.[37–39] However, recent studies found that after SCI, the latency of thermal nociceptive threshold increased in human patients and SCI injured mice.[20,40] The latter change could be caused by sensory deficits caused by damage to sensory pathways in the spinal cord or cauda equina.[40] Upon thermal stimulation of the hindlimb paws, the withdrawal latencies of the mild and moderate injury groups were significantly increased compared with the sham group. Our results indicate that a T10 contusion injury could damage sensory pathways,

resulting in increased thermal nociception threshold.[40] However, no significant difference was found between the mild and moderate injury groups. This indicates that the Hargreaves test is not sensitive enough to distinguish graded contusion injury in mice.

Our histological data showed that, when the injury severity increased, the lesion area increased and the spared white matter decreased at the injury epicenter. The correlations of the lesion area or spared white matter with the injury displacement were remarkably significant. These results were consistent with a previous LISA study on the rat SCI model.[13]

Conclusion

In conclusion, the LISA impactor is a laser-guided, displacement-based spinal cord contusive injury device that produces an accurate, graded, reliable, and reproducible contusive SCI at the thoracic level. Multiple functional and histological measurements can be used to assess functional and anatomical deficits associated with graded SCI produced by the LISA impactor for mice.

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Author Disclosure Statement

Dr. C.B. Shields holds an ownership position in LIS, Inc. The other authors have nothing to disclose.

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